REMARKS

Claims 1-38 are pending for examination. Applicant previously elected Group I (claims 1-26). Claims 1-26 are drawn to a method of expressing an immunotoxin in *Pichia pastoris* by methanol induction. Claim 1 is amended to recite "the immunotoxin," and "performing methanol induction on the *Pichia pastoris*," and "at a temperature below about 17.5°C". Claims 2 and 3 are amended to recite "methanol induction comprises a limited methanol feed." Claim 14 is amended to recite "a 4:1 methanol glycerol induction feed." New claim 39 is added herein with support as indicated below.

SPECIFICATION

The examiner has noted that the abstract of the disclosure exceeds 150 words. The abstract has been amended to be less than 150 words. The examiner has also stated that the "disclosure is objected to because it contains an embedded hyperlink and/or other form of browser-executable code on page 1, line 23." The disclosure has been amended so that the reference is no longer browser-executable.

SEQUENCE COMPLIANCE

The examiner notes that there are nucleotide and/or amino acid sequences in the specification that fail to recite a SEQ ID NO and therefore do not comply with the requirements of 37 C.F.R. § 1.821(d). Applicants note that the list of amino acids in Figure 1A, MQLCNITVWFYEA, is a list of individual possible mutations that His can be converted to (for reference see paragraph [0286] Kimata and Kohno JBC 1994;269:13497). MQLCNITVWFYEA is not a contiguous amino acid sequence; therefore, there is no requirement for it to be included in the Sequence Listing.

REJECTION UNDER 35 U.S.C. § 112, SECOND PARAGRAPH, INDEFITENESS

The examiner has stated that claim 14 is:

> rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claim 14 recites the limitation "the 4:1 methanol glycerol induction feed" in line 2. There is insufficient antecedent basis for this limitation in the claim.

Claim 14 has been amended to recite "a 4:1 methanol glycerol induction feed." This is believed to correct the issue due to antecedent basis.

REJECTION UNDER 35 U.S.C. § 112, FIRST PARAGRAPH, WRITTEN DESCRIPTION

Claims 10 is rejected as allegedly failing to comply with the written description requirement. The examiner has stated that:

> Claim 10 is rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.
>
> Claim 10 is drawn to a *Pichia* pastoris comprising a mutation in the amino acid sequence encoding EF-2. Therefore, applicants claim a genus of *Pichia* cells comprising mutant EF-2 sequences. The written description requirement for genus claims may be satisfied through sufficient description of a representative number of species by actual reduction to practice, reduction to drawings, or by disclosure of relevant identifying characteristics, i.e. structure or other physical and/or chemical properties, by functional characteristics coupled with known or disclosed correlations between function and structure, or by a combination of such characteristics sufficient to show that the applicant was in possession of the claimed genus.
>
> The specification discloses a single sequence ofEF-2 and that is represented by SEQ ID NO: 13. This sequence is used to generate a single species of cells wherein EF-2 is mutated so that the Gly amino acid at position 701 has been

changed to an Arg. This mutation results in a prevention of ADP-ribosylation of EF-2 in other organisms. Hence, applicants demonstrate a single species of cells and that is a cell in which EF-2 (SEQ ID NO:13) has a Gly to Arg mutation at position 701.

Applicants traverse. The application teaches numerous mutations in eukaryotic EF-2s that were already known in the art. As shown in the existing description of Figure 1A, there is a conserved region of the EF-2 protein sequence wherein mutations of the His or Gly have resulted in "EF-2s that were resistant to ADP-ribosylation" (see specification, paragraph [0076]). For example, Kimata and Kohno (JBC 1994;269:13497) mutagenized the conserved His to 19 other amino acids, and found 13 that resulted in functional *S. cerevisiae* EF-2 proteins that were not able to be ribosylated by diphtheria toxin when expressed in yeast. Furthermore, another DT-resistant strain producing non-ribosylatable EF-2s was isolated from cultured mammalian CHO cells and found to have a Glu to Arg mutation (Kohno and Uchida JBC 1987;262:12298). Because of this, one of skill would view applicants as in possession of the scope of EF-2 mutations that would allow for expression of an immunotoxin in *Pichia pastoris* (see Figure 1A and paragraph [0019], and see references in paragraph [0076]).

New claim 39 is added with support in claims 1, 10 and 6 as filed. This claim is believed to have written description in the specification for the reasons given above.

REJECTION UNDER 35 U.S.C. § 103

Claims 1,3,5-10, 12, 13, 15-20 and 22-25 are rejected as allegedly obvious over Madsen et al (US 6,723,536) in view of Neville et al (WO 01/87982). Also, Claims 2, 4, 11, 14,21 and 26 are rejected as allegedly obvious over Madsen et al (US 6,723,536) in view of Neville et al (WO 01/87982), and further in view of Magota et al (6,171,828) and McGrew et al (Gene, 1997,

Vol 187(2), pages 193-200) and Chang et al (US 6,992,172). The examiner has stated that:

> Claims 1,3,5-10, 12, 13, 15-20 and 22-25 are rejected under 35 U.S.c. 103(a) as being unpatentable over Madsen et al (US 6,723,536; see entire document) in view of Neville et al (WO 01/87982; see entire document).
>
> Applicants claim a method of expressing an immunotoxin in *Pichia pastoris* comprising growth in enzymatic digest of protein and yeast extract upon which methanol induction is performed at a temperature below 17.5°C.
>
> Neville et al teach expression of proteins in *Pichia pastoris* wherein growth in is enzymatic digest of protein and yeast extract which methanol induction. Growth media comprises 4% glycerol, *about* 2% yeast extract, 2% enzymatic digest of protein, 1.34% yeast nitrogen base with ammonium sulfate and without amino acids, .43% PTMI solution, wherein growth occurs at pH 3.5, and 0.01% antifoaming agent. Dissolved oxygen is about 40% (see figure 41). Methanol induction is performed at pH 7.0 wherein the agitation is 800 rpm (about 400 rpm) see page 159. Casamino acids and yeast extract serve as a source of amino acids and PMSF for at least 2 hours (it maintained expression level for **11** hours; see page 160, ¶ 2). Neville teach us of a mutant *Pichia pastoris* cell that comprises a mutation in the EF2 gene and is used for expression of A-dmDT390-bisFV(UCHTl) (see figure 20 and page 138, ¶ 4, page 55, line 1-5).
>
> Neville et al do not teach that the temperature is below 17.5°C.
>
> Madsen et al teach methods of producing recombinant proteins wherein *Pichia* cells are grown in media comprising enzymatic digestion of protein and yeast extract (see col 7-8). Methanol induction was performed wherein the induction was performed at less than 20°C and in some embodiments at 10°C (see col 7, line 35-44), which range encompasses 15°C. Glycerol containing media is fed to the glycerol containing cells and dissolved oxygen is 30% (see col 8, batch glycerol phase).
>
> Claims 2, 4, 11, 14,21 and 26 are rejected under 35 U.S.c. 103(a) as being unpatentable over Madsen et al (US 6,723,536; see entire document) in view of Neville et al (WO 01/87982; see entire document) as applied to claims 1,3,5-10, 12, 13, 15-20 and 22-25 above, and further in view of Magota et al (6,171,828; see entire document) and McGrew et al (Gene, 1997, Vol 187(2), pages 193-

> 200; see entire document) and Chang et al (US 6,992,172; see entire document).
>
> The teachings of Neville et al in view of Madsen et al are as above, except neither teaches specifically that methanol induction occurs by 1) limited methanol feed of 0.5- 0.75 ml/min/l0L or 2) a glycerol:methanol feed wherein the ratio of glycerol to methanol is 4: 1. Nor do any of the previously cited references teach use of soy digest of protein.

Applicants note that the examiner has stated that "Neville et al do not teach that the temperature is below 17.5°C." The examiner has also stated that in contrast Madsen et al teach that "[m]ethanol induction was performed wherein the induction was performed at less than 20°C and in some embodiments at 10°C (see col 7, line 35-44), which range encompasses 15°C."

Applicants respectfully disagree with the Examiner's characterization of the teaching of Madsen et al. More specifically, Madsen et al. do not recite a temperature for methanol induction. Rather, Madsen et al teach that at the harvest phase, in order "[t]o minimize foaming, the methanol and pH loops are not shutoff until the temperature is below 20°C" (see col. 7, lines 41 and 42). This does not explicitly teach the temperature at which the methanol induction step takes place. Furthermore, the teaching implies that the temperature of the methanol induction is above 20°C, and that it is shut off when it goes below 20°C. This is not supportive of an assertion that Madsen et al. teach a methanol induction step at below 20°C, much less at 17.5°C.

Additionally, harvesting is a discrete step with differing conditions that occurs after the methanol induction phase. In this regard Madsen et al. teach that harvest conditions are set (see col. 7, line 39) after completion of the methanol induction phase. In further support of this interpretation, a lower temperature is also first noted in the process data sheets under "Harvest conditions and Specifics" after the "Induction Phase" section (see col. 9, lines 24-34). Thus, the

reference to 10°C in Madsen et al. is directed to the harvest phase, not the methanol induction phase. Thus, Madsen et al. at most teach a methanol induction phase that ceases at 20°C, with no evidence that it continues at any temperature lower than that. That is, there is no teaching in Madsen et al. of methanol induction at below about 17.5°C.

As such, the combination of Madsen et al. and Neville et al. fails to suggest "performing methanol induction on the *Pichia pastoris*, wherein the methanol induction is at a temperature below about 17.5°C" as is provided in claims 1-24, and separately in claims 25 and 26. Such claims are, therefore, not obvious over the cited art.

Moreover, applicants respectfully submit that the claims are also not obvious because of the fact that the applicants have experienced a significant improvement in yield using a lower temperature for the methanol induction—a result that is surprising. In Figure 21D and 21E it can be seen that the yield is more than doubled when the induction temperature is changed from 23-25°C to 15°C (see page 3, paragraph [0039] for Figure legend). Surprising results are necessarily non-obvious. Thus, not only is the section 103 rejection of claims 1-27 not legally sufficient, it is not supported by the facts of record.

As such, Applicant believes the rejection to be overcome and respectfully request its withdrawal.

CONCLUSION

In view of the above amendments and remarks, reconsideration and allowance of the application are believed to be merited and are respectfully requested. The Examiner is invited to directly contact the undersigned if doing so will expedite the prosecution of this application.

The fee of $50.00 for an additional claim in excess of 20 is due in connection with this submission. This fee is believed to be correct. However, if a fee is due, the Commissioner is hereby authorized to charge any such fee or credit any overpayment to Deposit Account No. 14-0629.

Respectfully submitted,

NEEDLE & ROSENBERG, P.C.

Gwendolyn D. Spratt
Registration No. 36,016

NEEDLE & ROSENBERG, P.C.
Customer Number 36339
(678) 420-9300
(678) 420-9301 (fax)

CERTIFICATE OF ELECTRONIC TRANSMISSION UNDER 37 C.F.R. § 1.8			
I hereby certify that this correspondence, including any items indicated as attached or included, is being transmitted via electronic transmission via EFS-Web on the date indicated below.			
Name of Person Mailing (Print/Type)	Gwendolyn D. Spratt		
Signature	Gwendolyn D. Spratt	Date	5-28-08